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Rapid metabolic adaptation in European sea bass (*Dicentrarchus labrax*) juveniles fed different carbohydrate sources after heat shock stress

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Abstract:

A study was conducted to evaluate the effect of two dietary carbohydrate sources (waxy maize starch and glucose) on the metabolic adaptation of sea bass juveniles (initial weight: 24 g) to a heat shock treatment (temperature rise from 18 °C to 25 °C within 24 h). Two isonitrogenous and isolipidic diets were formulated to contain 20% waxy maize starch (WS diet) or 20% glucose (GLU diet). Triplicate groups of fish were fed to near satiation for 4 weeks at both temperatures (18 °C and 25 °C). Then, fish previously maintained at 18 °C were submitted to a heat shock (18 °C to 25 °C) and continued to be fed with the same diets during 1 more week. The higher water temperature significantly improved growth performance, feed efficiency, as well as protein efficiency ratio, independently of diet. At 25 °C, but not at 18 °C, growth of fish fed the WS diet was higher than that of fish fed the GLU diet. Plasma glucose levels were higher in sea bass fed the GLU diet and not influenced by water temperature. Fish fed a glucose diet or reared at high temperatures (25 °C) showed enhanced liver glycolytic, lipogenic and gluconeogenic capacities compared to fish fed a starch diet or reared at low temperatures (18 °C). For the majority of the enzymes studied, 1 week seemed to be enough time for metabolic adaptation in sea bass submitted to an acute heat shock.

Irrespective of carbohydrate source, HSP70 gene expression was similar in both cold water (18 °C) and warm water (25 °C) acclimated sea bass. A weak down regulation was observed after heat shock only in fish fed the GLU diet. This suggests that HSP70 gene expression is not affected by the rearing temperature per se.

Keywords: Biomarker; Carbohydrate utilization; European sea bass; Gluconeogenic enzymes; Glucose; Glycolytic enzymes; HSP70; Lipogenic enzymes; Temperature; Waxy maize starch

1. Introduction

Carnivorous fish require high-protein diets to obtain amino acids for use in protein synthesis, glucose synthesis and for energy purposes (Sánchez-Muros et al., 1998). However, protein is one of the most expensive components of diets and excess protein increases N excretion. Therefore, both from an economical and an environmental point of view, it is advisable to spare protein for plastic purposes by increasing the utilization of conventional energy sources like lipids or carbohydrates (Cho and Kaushik, 1990; Kaushik and Médale, 1994).

Although carbohydrates are the cheapest energy source, most teleosts do not tolerate high dietary carbohydrate levels, and maximum dietary inclusion level depends on fish species. It is generally assumed that optimal dietary digestible carbohydrate level is less than 20% for carnivorous fish, whereas it is much higher (30-40%) for omnivorous fish (Wilson, 1994). For European sea bass, there is evidence that a dietary incorporation of 20-25% of digestible carbohydrate does not affect growth or feed efficiency (Gouveia et al., 1995; Lanari et al., 1999; Peres and Oliva-Teles, 2002).

The complexity of carbohydrate molecule also affects carbohydrate utilization. For example, starch has been demonstrated to be used more efficiently than glucose in both marine and fresh water species: carp, red sea bream (Furuichi and Yone, 1982), yellowtail (Furuichi et al., 1986), tilapia (Anderson et al., 1984; Shiau and Peng, 1993), white sturgeon (Deng et al., 2005). On the contrary, juvenile grass carp (Tian and Liu, 2004) and rainbow trout (Bergot, 1979a; Hung and Storebakken, 1994) appears to utilise glucose better than starch. However, also in rainbow trout (Pieper and Pfeffer,

1980a; 1980b) and in grouper (Shiau and Lin, 2001) starch seems to be used as efficiently as glucose.

Various factors affect the digestible energy provided by complex carbohydrates in fish diets (Bergot, 1993). One of these factors is the technological treatment of starch. Contrary to normal maize, which contains 25-28% amylose, waxy maize only contains 1% (Pfeffer et al., 1991; Bergot, 1993). Bergot (1993) measured the digestibility of starch of different botanical origins by rainbow trout and found that digestibility of waxy maize starch was significantly higher than that of amylomaize or normal maize. Pfeffer et al. (1991) also found that rainbow trout performed better with waxy than normal maize starch.

For European sea bass, data on the utilization of different carbohydrate sources is still limited. Alliot et al. (1979) observed a protein sparing effect of glucose, although growth depression occured at high dietary inclusion levels. Despite differences in carbohydrate digestibility, Alliot et al. (1984) observed similar growth performance of sea bass fed diets including either maltose or starch. Although the effect of starch gelatinization on growth performance is somewhat discordant (Gouveia et al., 1995; Dias et al., 1998; Peres and Oliva-Teles, 2002) it is well established that gelatinization of starch improves carbohydrate digestibility (Dias et al., 1998; Peres and Oliva-Teles, 2002). Also in sea bass, digestibility of waxy maize starch was higher than that of normal maize starch (Enes et al., in press).

Water temperature is an important environmental factor affecting physiological and biochemical functions in fish (Jobling, 1994), as well as the activities of several hepatic enzymes (Shikata et al., 1995). In sea bass, temperature plays an important role in governing growth via its effects on feeding rate and metabolism (Person-Le Ruyet et al., 2004). The increase of water temperature from 18ºC to 25ºC improved growth and feed efficiency of sea bass juveniles (Peres and Oliva-Teles, 1999; Person-Le Ruyet et al., 2004). Although protein utilization seems to be affected by temperature (Peres and Oliva-Teles, 1999) the optimum dietary protein inclusion level is independent of water temperature (Alliot et al., 1974; Hidalgo and Alliot, 1988; Peres and Oliva-Teles, 1999).

Heat shock proteins (HSP) are a wide family of conserved proteins, classified according to their molecular weight, present in all organisms including fish (Basu et al., 2002). HSP70 is known to assist the folding of nascent polypeptide chains, act as a molecular chaperone, and mediate the repair and degradation of altered or denatured proteins (Basu et al., 2002). Thus, HSP70 has been most widely used as a biomarker of stress. In fish, like in mammals, HSP70 is induced by heat and chemical shocks (Gornati et al., 2004). In sea bass, HSP70 was also shown to be inducible by rearing density (Gornati et al., 2004).

According to our knowledge, there are no previous studies with sea bass comparing starch and glucose utilization at different water temperatures. Thus, the purpose of the present study was to evaluate the effect of two water temperatures (18ºC and 25ºC) and two carbohydrate sources (waxy maize starch and glucose) on growth performance and activities of hepatic enzyme related to glycolysis, gluconeogenesis and lipogenesis in European sea bass juveniles.

2. Material and methods

2.1. Diets

Two isonitrogenous (48% crude protein) and isolipidic (18% crude lipids) diets were formulated to contain 20% of either waxy maize starch (diet WS) or glucose (diet GLU). Waxy maize starch (99% amylopectin, 1% amylose) was purchased from Cerestar (Mechelen, Belgium) and D(+)-Glucose from MERCK. All dietary ingredients were finely ground, mixed thoroughly and dry pelleted in a laboratory pellet mill (CPM) through a 3 mm die. Ingredients and proximate composition of the experimental diets are presented in Table 1.

2.2. Fish rearing

Fish used in this study were European sea bass (*Dicentrarchus labrax*) juveniles obtained from a commercial hatchery. The first part of the trial lasted 4 weeks and was performed in two independent partial water recirculation systems, thermoregulated to 18.3±0.5ºC and 25.0±0.2ºC, respectively. Both systems contained a battery of 6 cylindrical fiberglass tanks of 250 L capacity each. Tanks were supplied with a continuous flow of filtered seawater (6.0 L min^{-1}), salinity averaged 37.3±0.5‰ and dissolved oxygen was kept near to satiation.

Fish were allocated to each system and acclimatized for 15 days to the tanks and water temperatures. Thereafter, 30 fish with an average body weight of 24g were randomly distributed to each tank. Each diet was assigned to triplicate groups of animals. Fish in both water systems were fed by hand twice a day, six days a week, to near satiation with one of the experimental diets during 4 weeks. Then, fish were bulk weighed and 5 fish from each tank were sampled for determination of hepatosomatic

and visceral indices (sampling #1). In order to minimise stress caused by manipulation, the remaining fish were fed for 3 more days and then blood and liver from 6 fish per tank were sampled six hours after the morning meal (sampling #2).

Thereafter, the water temperature of the 18ºC system was quickly raised (within 24 hours) to 25ºC and fish continued to be fed to near satiation with the same diets during 1 more week. Then, blood and liver from 6 fish per tank submitted to the temperature change were also sampled six hours after the morning meal (sampling #3).

Blood was collected from the caudal vein, immediately centrifuged and the plasma frozen at -20ºC for later analysis. After collection, livers were quickly frozen in liquid nitrogen and then stored at –80°C until measurement of enzymatic activities and heat shock protein 70 (HSP70) gene expression.

2.3. Analytical methods

2.3.1. Proximate analysis

Chemical analysis of the diets was conducted using the following procedures: dry matter after drying at 105°C until constant weight; ash by combustion in a muffle furnace at 450°C for 16h; protein content (N × 6.25) by the Kjeldahl method after acid digestion using Kjeltec digestion and distillation units; lipid by petroleum ether extraction (Soxtec HT System) and gross energy by direct combustion in an adiabatic bomb calorimeter (PARR model 1261). Plasma glucose was determined using an enzymatic-colorimetric method (glucose kit, cod. 1001191; Spinreact).

2.4. Enzyme activity

2.4.1. Hexokinase /glucokinase and pyruvate kinase activities

In order to measure hexokinase (HK; EC 2.7.1.1) / glucokinase (GK; EC 2.7.1.11) and L-type pyruvate kinase (PK; EC 2.7.1.40) activities, a frozen sample of liver (200mg) was homogenized (dilution 1 / 10) in ice-cold buffer (80mM Tris; 5mM EDTA; 2mM DTT; 1mM benzamidine; 1mM 4-(2-aminoethyl) benzenesulfonyl fluoride, pH 7.6). After centrifugation (900× *g* for 10 min), the resultant supernatant was separated for HK/GK and PK activity measurements. The HK (low Km HKs) and GK (high Km HK or HK IV) activities were measured using 0.5 mM and 100 mM of glucose, respectively, as described previously (Tranulis et al., 1996; Panserat et al., 2000a) at 37°C by coupling ribulose-5-phosphate formation from glucose-6-phosphate to the reduction of β-NADP using purified glucose-6-phosphate dehydrogenase (Sigma) and 6-phosphogluconate dehydrogenase (Sigma) as coupling enzymes. This assay for measuring GK activity on frozen samples necessitated correction by measuring glucose dehydrogenase (EC 1.1.1.47) activity as described by Tranulis et al. (1996). To measure PK activities, the supernatant was centrifuged at 10 000× *g* for 20 min and the resultant cytosolic fraction was used for enzyme activity measurements. The procedure followed was that of Foster and Moon (1985), monitoring the decrease in absorbance at 340 nm (β-NAD, reduced form disappearance) using purified lactate dehydrogenase (Sigma) in excess as the coupling enzyme.

2.4.2. Fructose-1,6-bisphosphatase activities

To measure the activity of fructose-1,6-bisphosphatase (FBPase; EC 3.1.3.11), a frozen sample of liver (200mg) was homogenized (dilution 1 / 10) in ice-cold buffer (20mM Tris; 5mM EDTA; 2mM DTT; 0.24mM saccharose, pH 8). The homogenate was centrifuged at 900× *g* for 10 min and the resultant supernatant was centrifuged at 10 000× *g* for 20 min. Enzyme assays were performed on cytosolic fractions as previously

described by Tranulis et al. (1996), monitoring the increase in absorbance (β-NADPH appearance) using purified glucose-6-phosphate dehydrogenase (Sigma) and 6-phosphogluconate dehydrogenase (Sigma) as coupling enzymes.

2.4.3. Glucose-6-phosphatase activities

In order to measure glucose-6-phosphatase (G6Pase; EC 3.1.3.9) activity, microsomes were obtained from sea bass livers, as described previously (Panserat et al., 2000b). Microsomes were suspended in buffer (100mM NaH_2PO_4; 25mM Na_2HPO_4; 2mM EDTA; 1mM DTT, pH 7), without further treatment. The procedure followed was that of Alegre et al. (1988), monitoring the increase in absorbance at 340 nm (β-NADH appearance) using purified glucose dehydrogenase (Sigma) in excess as the coupling enzyme.

2.4.4. Glucose-6-phosphate dehydrogenase activities

For measurement of glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) activity, a frozen sample of liver (200 mg) was homogenized (dilution 1 / 5) in ice-cold buffer (0.02M Tris; 0.25M sucrose; 2mM EDTA; 0.1M NaF; 0.5mM PMSF; 0.01M β-mercapto ethanol, pH 7.4). Homogenates were centrifuged at 30 000x *g* for 20 min. Enzyme assays were performed as previously described by Bautista et al. (1988).

2.4.5. Specific enzyme activities

All enzyme activities were expressed per mg of hepatic soluble protein (specific activity). Protein concentration was determined according to Bradford (1976) using a Sigma protein assay kit with bovine serum albumin as a standard. One unit of enzyme

activity was defined as the amount of enzyme that catalysed the hydrolysis of 1 µmol of substrate per min under the standard conditions (37°C).

2.5. *HSP70 gene expression analysis: real-time polymerase chain reaction (PCR)*

Total RNA was extracted from sea bass livers using TRIzol reagent (Invitrogen, Carlsbad, CA) and HSP70 mRNA levels were determined by real-time RT- PCR. RNA samples were treated by RQ1 RNase-Free DNase prior to RT-PCR (Promega, Madison, WI), to avoid genomic DNA amplification. cDNA was generated from 1µg DNase-treated RNA using SuperScript ™ III RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA). Real-time PCR was performed in the iCycler iQ ™ (BIO-RAD, Hercules, CA). Quantitative PCR analyses for HSP70 were performed on 10 µl of the diluted RT reaction mixture using the iQ ™ SYBR ® Green Supermix (BIO-RAD, Hercules, CA). The total volume of the PCR reaction was 25 µl, containing 200 nM of sea bass gene specific primers (Table 2). Thermal cycling was initiated with the incubation at 95°C for 3 minutes for hot-start iTaq ™ DNA polymerase activation. 35 steps of PCR were performed, each one consisting of heating at 95°C for 20s for denaturing, and at 59°C for 30s for annealing and extension. Following the final cycle of the PCR, melting curves were systematically monitored (temperature gradient at 0.5°C/10s from 55°C up to 94°C). Relative quantification of the target gene transcript (HSP70) with a chosen reference gene transcript (the sea bass elongation factor 1α (EF1α), previously used in another fish species, rainbow trout (Gabillard et al., 2003), was made following the Pfaffl method with the Relative Expression Software tool (REST©) (Pfaffl, 2001; Pfaffl et al., 2002). This mathematical algorithm, which needs no calibration curve, computes an expression ratio, based on real-time PCR efficiency and the crossing point deviation of the sample *versus* a control:

$$R = \frac{[(E_{HSP70})^{\Delta CT_{HSP70}\,(\text{mean control - mean sample})}]}{[(E_{EF1\alpha})^{\Delta CT_{EF1\alpha}\,(\text{mean control - mean sample})}]}$$

where E is PCR efficiency determined by standard curve using serial dilution of cDNA; ΔCT the crossing point deviation of the sample *versus* a control.

2.6. Statistical analysis

Data are presented as means ± standard deviation. Statistical evaluation of the results was done by one-way or two-way analysis of variance using a Statgraphics Plus Version 7.0 for Windows software package. Before analysis, hepatosomatic index, GK, and PK activities were log transformed for normality. A probability level of 0.05 was used for rejection of the null hypothesis. Significant differences among means were determined by the Tukey's multiple range test and by the non parametric Rest statistical test for HSP70 gene expression analysis.

3. Results

Both feed intake and growth were significantly higher in fish held at the higher water temperature (Table 3). At 25ºC, final body weight and specific growth rate were significantly higher in fish fed diet WS than diet GLU, whereas at 18ºC there were no significant differences between fish fed the two experimental diets. Both feed intake (g kg ABW^{-1} day^{-1}) and feed efficiency were significantly higher at 25ºC than at 18ºC (Table 3). Within each temperature, feed intake was significantly higher in fish fed diet WS while feed efficiency was significantly higher in fish fed diet GLU. Protein efficiency ratio (PER) was also significantly higher in fish held at 25ºC (Table 3). Nevertheless, within each temperature there were no significant differences in PER among groups. While at 25ºC the visceral index (VI) was unaffected by diet composition, at 18ºC it was significantly higher in fish fed GLU diet (Table 3). Hepatosomatic index (HSI) was significantly higher in fish held at the lower water temperature (Table 3). Within each temperature, HSI was higher in fish fed GLU diet. Plasma glucose was not significantly affected by water temperature. It was, however, higher in fish fed GLU diet than WS diet and this difference was significant at 25ºC (Table 3). The quick rise of water temperature from 18ºC to 25ºC had no significant effect on plasma glucose levels (data not shown).

Within each temperature, there were no significant differences in HK and G6Pase activities among groups (Table 4). There was also no effect of temperature on HK and G6Pase activities. On the contrary, significant variations were noticed for the other enzymes studied. Indeed, GK, FBPase and G6PD activities were significantly higher at 25ºC than at 18ºC (Table 4) while there was no effect of temperature on PK

(Table 4). Within each temperature, GK, PK and G6PD activities were significantly higher in fish fed GLU diet while FBPase activity was higher in fish fed WS diet, although this latter difference was only significant in fish kept at 18ºC.

The effect of a rise in water temperature from 18ºC to 25ºC on hepatic glycolytic/gluconeogenic and lipogenic enzyme activities is present in Fig. 1. In fish fed both diets and submitted to temperature shock, activities of HK, GK, FBPase and G6Pase were not different from those of groups maintained at 25°C for the whole period. PK activity significantly increased after the heat shock in fish fed both diets and was significantly higher than in fish previously acclimated to 25ºC. G6PD activity in fish fed diet GLU, but not diet WS, was significantly lower in the group submitted to the heat shock in comparison to the 25ºC acclimated fish.

In fish fed diet WS, there were no differences in hepatic HSP70 gene expression (Table 5). In contrast, in fish fed diet GLU, even though there were also no differences in hepatic HSP70 gene expression between fish raised at 25°C and at 18ºC (Table 5), HSP70 gene expression was found to be lower in fish submitted to the temperature shock (18°C to 25°C) in comparison to fish acclimated to 25ºC or 18°C, respectively.

4. Discussion

Irrespective of carbohydrate source, we found that HSP70 gene expression was similar in both cold water (18ºC) and warm water (25ºC) acclimated sea bass, which suggests that HSP70 gene expression is not associated to the rearing temperature *per se*. In contrast, in silver sea bream, Deane and Woo (2005) observed that acclimation to cold temperature (12ºC) during one month resulted in an up regulation of HSP70 in comparison to fish at 25ºC. However, one week after heat shock (18°C to 25°C) a down-regulation of HSP70 gene expression in comparison to fish acclimated to 25ºC or 18ºC was observed in fish fed the GLU diet (but not with the WS diet for unknown reasons). Indeed, Ojima et al. (2005) and Cara et al. (2005) observed also a variation of HSP70 gene expression after an increase of temperature in trout cells and gilthead seabream respectively. Thus, our data suggest that HSP70 may be a good bio-marker in sea bass, as in other species, for an acute modification of rearing temperature. We discuss now the effects of water temperature on growth and metabolism in European sea bass.

As expected, and irrespective of carbohydrate source, results of the present study showed that the increase of water temperature (18°C vs. 25°C) significantly improved growth performance, feed efficiency, as well as protein efficiency ratio in sea bass juveniles. Similar results were already reported for this species (Alliot et al., 1983; Hidalgo et al., 1987; Peres and Oliva-Teles, 1999; Person-Le Ruyet et al., 2004) and for other species such as trout (Cho and Slinger, 1979; Cho and Watanabe, 1985; Alanara, 1994; Capilla et al., 2003) and carp (Goolish and Adelman, 1984).

At 25ºC, growth of fish fed the starch diet was better than that of fish fed the glucose diet. At 18ºC such a difference was not evident due to the poor growth of both groups at that temperature. The poor ability to utilize simple carbohydrates was also observed in other fish species such as tilapia (Anderson et al., 1984; Shiau and Peng, 1993), sunshine bass (Hutchins et al., 1998), common carp and red sea bream (Furuichi and Yone, 1982). Dietary glucose is rapidly absorbed across the gut comparatively to complex carbohydrates such as starch (Lin and Shiau, 1995). It has been suggested that in fish fed glucose rich diets a considerable amount of glucose may enter the blood before adequate insulin levels are available to allow its utilization at cellular level (Pieper and Pfeffer, 1980a; Furuichi and Yone, 1981). Complementary data on plasma insulin profiles in European sea bass might be of use.

Several authors have observed that yellowtail (Furuichi, 1983), hybrid tilapia (Shiau and Peng, 1993), Indian major carp (Erfanullah and Jafri, 1995), common carp (Shikata et al., 1994), grouper (Shiau and Lin, 2001) and silver perch (Stone, 2003) utilize complex carbohydrates, such as gelatinized starch or dextrin, more efficiently than glucose to spare protein for growth. But, in the present study, data on protein efficiency ratio suggest that in sea bass there is a similar protein sparing effect of both carbohydrates, irrespective of water temperature.

Plasma glucose levels six hours after feeding were affected by dietary carbohydrate source. Indeed, plasma glucose concentration was higher in sea bass fed diet GLU than diet WS, and this difference was significant at 25ºC. Similar results regarding carbohydrate source were previously observed in rainbow trout (Bergot, 1979b; Brauge et al., 1994) and in tilapia (Shiau and Liang, 1995). However, contrary to

what was reported for rainbow trout (Brauge et al., 1995), we did not found any specific effect of temperature on plasma glucose levels. Thus, carbohydrate source seems to be the major factor controlling glycaemia values in sea bass.

At both water temperatures, HK activity was unaffected by carbohydrate source, confirming that also in European sea bass this enzyme is not under nutritional regulation like in rainbow trout, gilthead seabream, common carp (Panserat et al., 2000a) and hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) (Lin and Shiau, 1995). Nevertheless, we observed that HK activity is lower when compared with activities found in other finfish fed similar level of carbohydrates (Panserat et al., 2000a; Kirchner et al., 2003a). HK activity was also unaffected by rearing temperature as well as by heat shock as was previously observed in rainbow trout by Tranulis et al. (1991). However, also in rainbow trout, a higher activity of HK at 18ºC than at 8ºC was recently reported (Capilla et al., 2003).

Irrespective of water temperature, dietary glucose does appear to induce GK and PK more than starch. This may be related to the higher glycaemia in fish fed diet GLU, which may possibly induce the liver capacity to store excess glucose as it was observed in rainbow trout (Panserat et al., 2001; Kirchner et al., 2003b). In fact, HSI was higher in fish fed GLU diet which may be due to higher glycogen content. There was also a positive effect of temperature on GK activity but not on PK. Moreover, after the heat shock, GK activity quickly raised to values previously found at 25°C indicating that there was a rapid adaptation of liver glucose metabolism to the higher feed intake, and consequent increased glucose supply. In contrast, in response to heat shock, PK activities raised to values significantly higher than those previously found at 25ºC,

suggesting a transitory compensation or up-regulation associated with a temporary metabolic reorganization in the fish (Tranulis et al., 1991).

Data on the effect of temperature on hepatic gluconeogenesis in fish are scarce and discordant (Woo, 1990; Shikata et al., 1995).We found here, a significant effect of carbohydrate source and water temperature on the activity of FBPase but not that of G6Pase. In fact the starch-based diet led to higher FBPase activity than the glucose diet, in contrast to what was previously reported for hybrid tilapia (Shikata et al., 1994). Moreover, FBPase activity was significantly higher in the warm-acclimated fish suggesting an enhanced gluconeogenic activity at higher temperature. After the heat shock, FBPase activity was not significantly different from values found in sea bass previously reared at 25ºC, suggesting that for FBPase, as it was observed for GK, one week was long enough for a metabolic reorganization in relation to temperature change.

In the present study, and irrespective of water temperature, G6PD activity in sea bass fed the glucose diet was significantly higher than in fish fed the starch diet. This may be associated to the higher glycemia observed in this group which enhanced lipogenesis. Similar results were already reported in sturgeon (Hung et al., 1989), rainbow trout (Hung and Storebakken, 1994) and common carp (Shikata et al., 1994). While rearing temperature had no effect on G6PD activity in fish fed diet WS, in fish fed diet GLU, G6PD activity was significantly higher in the warm-acclimated fish, indicating a higher lipogenic activity at higher temperatures in this group. This may be due to the higher feed intake (i.e. glucose) recorded in fish reared at 25ºC. Tranulis et al. (1991) also reported no differences in G6PD activity of rainbow trout reared at 0-2ºC or at 10-17ºC and fed a 16% carbohydrate diet. One week after the heat shock G6PD

activity remained similar to that observed in fish reared at 18ºC. This suggests a longer adaptation period to water temperature changes for G6PD as compared to glycolytic (GK and PK) and gluconeogenic (FBPase) enzymes.

In conclusion, carbohydrate source and water temperature are important factors in regulation of carbohydrates metabolism in sea bass juveniles. Thus, fish fed a glucose diet or reared at high temperatures (25ºC) presented enhanced liver glycolytic, lipogenic and gluconeogenic capacities compared to fish fed a starch diet or reared at low temperatures (18ºC). Overall, the present data showed that one week seems to be sufficient for a metabolic reorganization to occur in sea bass submitted to a sudden rise in temperature of 7ºC in 24h.

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Table 1
Composition and proximate analyses of the experimental diets

	Diets	
	WS	GLU
Ingredients (% dry weight)		
Fish meal[a]	58.7	58.7
Soluble fish protein concentrate[b]	5.0	5.0
Cod liver oil	11.9	11.9
Waxy maize starch[c]	20.9	-
D-Glucose	-	20.9
Vitamin premix[d]	1.0	1.0
Mineral premix[e]	1.0	1.0
Choline chloride (60%)	0.5	0.5
Carboxymethylcellulose	1.0	1.0
Proximate analyses (% dry weight)		
Dry matter	92.3	90.2
Crude protein	47.2	49.4
Crude fat	17.7	17.7
Ash	11.4	12.1
Gross energy (kJ g^{-1} DM)	22.4	23.1

[a]Pesqueira Diamante, Steam Dried LT, Spain (CP: 75.3% DM; GL: 8.8% DM).
[b]Sopropèche G, France (CP: 75.4% DM ; GL : 19.2%DM).
[c]Cerestar (Mechelen, Belgium).
[d]Vitamins (mg Kg^{-1} diet): retinol acetate, 18 000 (IU Kg^{-1} diet); cholecalciferol, 2 000 (IU Kg^{-1} diet); alpha tocopherol acetate, 35; sodium menadione bisulphate, 10; thia - min-HCl, 15; riboflavin, 25; calcium pantothenate, 50; nicotinic acid, 200; pyridoxine HCl, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbic acid, 50; inositol, 400.
[e]Minerals (mg Kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate 200; sodium fluoride, 2.21; potassium iodide; 0.78; magnesium oxide; 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 5.93 (g Kg^{-1} diet); potassium chloride, 1.15 (g Kg^{-1} diet); sodium chloride, 0.40 (g Kg^{-1} diet).

Table 2

Nucleotides sequences of the PCR primers used to assay gene expression by real-time quantitative PCR

	Forward primer	Reverse primer	Annealing temperature (ºC)	PCR produt length (pb)
HSP70	gtctggacaaaggcaagagc	cacaaagtggttgaccatgc	55°C	176
EF1α	cccctggacacagagacttc	ttgactccaacgatgagctg	54°C	184

Sea bass HSP70 gene - genbank accession number: AY423555

Sea bass EF1α gene - genbank accession number: AJ866727

Table 3
Growth performance, feed utilization efficiency, visceral and hepatic index and plasmatic glucose of sea bass fed the experimental diets during 4 weeks (Initial VI = 10.5; Initial HSI = 1.5)

Temperature	25ºC		18ºC	
Diets	WS	GLU	WS	GLU
Initial body weight (IBW), g	24.0	24.0±0.02	24.0	24.0
Final body weight (FBW), g	39.2±0.4[a]	36.2±0.8[b]	30.3±0.8	29.7±1.0
Feed intake (g kg ABW^{-1} day^{-1})	21.3±0.4[a]	16.5±1.1[b]	13.3±0.8[a]	10.2±0.8[b]
Specific growth rate (%)[1]	1.8±0.04[a]	1.5±0.08[b]	0.8±0.09	0.8±0.1
Feed efficiency[2]	0.8±0.01[a]	0.9±0.03[b]	0.6±0.04[a]	0.7±0.06[b]
Protein efficiency ratio[3]	1.7±0.03	1.8±0.06	1.3±0.09	1.5±0.13
VI[4]	10.4±1.4	11.3±1.5	10.3±1.3[a]	11.9±2.1[b]
HSI[5]	1.5±0.3[a]	2.4±0.5[b]	2.1±0.4[a]	3.4±1.0[b]
Plasma glucose (mmol l^{-1})	6.9±1.7[a]	9.5±1.6[b]	8.9±3.8	11.8±4.7

Two-way ANOVA

Variation source[6]	Diet	Temperature	Interaction
Final body weight (FBW), g	**	***	*
Feed intake (g kg ABW^{-1} day^{-1})	***	***	ns
Specific growth rate (%)	**	***	ns
Feed efficiency	**	***	ns
Protein efficiency ratio	*	***	ns
VI	**	ns	ns
HSI	***	***	ns
Plasma glucose (mmol l^{-1})	*	ns	ns

ABW: average body weight (initial body weight + final body weight) / 2.
Values are means ± S.D.. At each temperature, means in the same line with different superscript letters are significantly different (Tukey test, P<0.05).
[1]Specific growth rate: ((ln(FBW)-ln(IBW) / (time in days)) x 100.
[2] Feed efficiency: wet weight gain / dry feed intake.
[3]Protein efficiency ratio: wet weight gain / crude protein intake.
[4] VI Visceral index: (viscera weight/body weight) x 100.
[5] HSI Hepatossomatic index: /liver weight/body weight) x 100.
[6] *P<0.05; **P<0.01; ***P<0.001; ns: non-significant.

Table 4
Hepatic glycolytic (hexokinase, glucokinase and pyruvate kinase), gluconeogenic (fructose-1,6-bisphosphatase and glucose-6-phosphatase) and lipogenic (glucose-6-phosphatase dehydrogenase) enzymes activities (mU/mg protein) in sea bass fed the experimental diets during 4 weeks

Temperature	25ºC		18ºC	
Diets	WS	GLU	WS	GLU
Glycolysis				
Hexokinase	0.53±0.47	0.59±0.24	0.38±0.33	0.56±0.41
Glucokinase	2.6±1.3[a]	13.1±7.9[b]	1.7±1.1[a]	4.3±3.2[b]
Pyruvate Kinase	66.7±9.1[a]	110.3±26.2[b]	68.9±10.8[a]	90.0±13.3[b]
Gluconeogenesis				
Fructose-1,6-bisphosphatase	28.0±3.7	24.6±3.7	23.5±3.5[a]	20.1±2.7[b]
Glucose-6-phosphatase	15.5±2.4	16.3±3.4	16.8±4.8	16.1±2.9
Lipogenesis				
Glucose-6-phosphate dehydrogenase	152.3±18.0[a]	257.7±19.6[b]	151.8±30.0[a]	212.1±44.2[b]

Two-way ANOVA

Variation source[1]	Diet	Temperature	Interaction
Glycolysis			
Hexokinase	ns	ns	ns
Glucokinase	***	**	ns
Pyruvate Kinase	***	ns	ns
Gluconeogenesis			
Fructose-1,6-bisphosphatase	**	***	ns
Glucose-6-phosphatase	ns	ns	ns
Lipogenesis			
Glucose-6-phosphate dehydrogenase	***	*	*

Values are means±S.D. Significant differences within the diets are present by different letters (Tukey test, P<0.05).

[1] * P<0.05; ** P<0.01; *** P<0.001; ns: non-significant.

Table 5

Expression of hepatic HSP70 gene in sea bass reared at different temperatures

Temperature	Diets	Type of regulation		p value
(control temperature: 25ºC)				
18ºC	WS	no difference		0.708
	GLU	no difference		0.571
18ºC to 25ºC	WS	no difference		0.535
	GLU	down-regulation	1.2-fold	0.021
(control temperature: 18ºC)				
18ºC to 25ºC	WS	no difference		0.776
	GLU	down-regulation	1.3-fold	0.003

EF1α gene expression – no variation

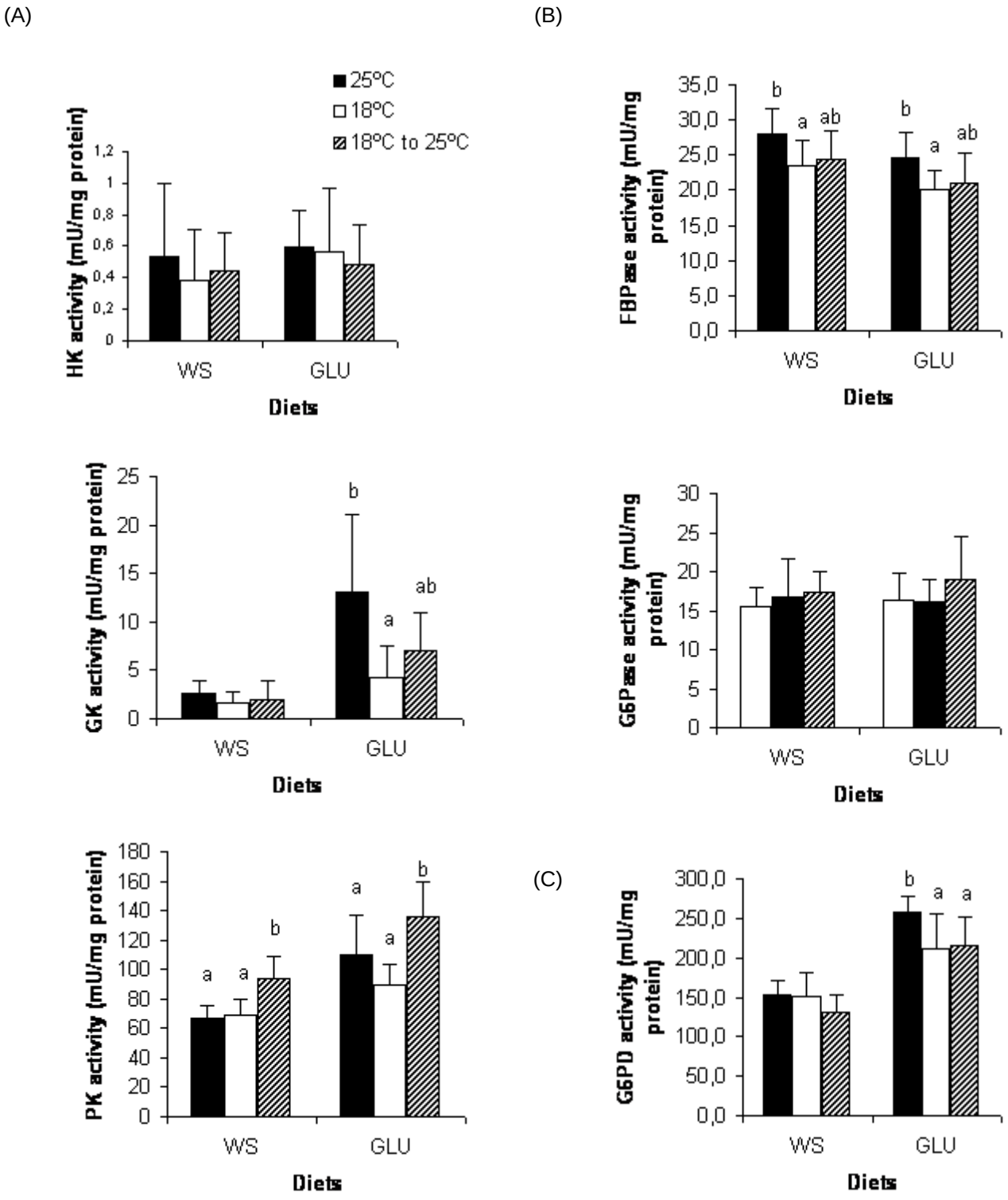

Fig. 1. Effect of water temperature on hepatic glycolytic (A), gluconeogenic (B) and lipogenic (C) enzyme activities in fish fed with waxy starch (WS) and glucose diet (GLU). At each diet, means are significantly different when they are represented by by different letters (Tukey test, $P < 0.05$).